ulated" matrix cells (= melanophores) exhibit characteristic dense pigmentation. The keratinized hair shaft arises from these matrix cells. Pigmented matrix cells seldom divide, but new cells arise from the proliferating lower bulb and receive pigment as they pass by the melanoblast processes of the upper bulb. As few as 4 melanoblasts have been observed to supply the stream of matrix cells of a small follicle (zigzag) and the presence of only one or two results in a mosaic.

From histological and x-radiation evidence it appears that the "reservoir" of melanoblasts is within the epidermal follicle (cf. Taylor, 1949). Dermal melanophores apparently do not contribute to pigmentation in the hair follicle. The epidermal melanoblasts are the original granule-forming cells, but in the recipient matrix cells, the characteristic shape and color of the phenotype are further imposed upon the granules. Whereas biotin deficiency, for instance, causes a failure of melanin-formation on these granule sites (Chase and Rauch, in press), x-radiation destroys the source of supply of granules, namely, the epidermal melanoblasts of the follicle.

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A Comparative Colorimetric Study of Dopa-melanin Formation by Melanomas and Pigmented Skins.

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The costo-vertebral pigmented skin spot of the Syrian hamster, which contains both intradermal dentritic pigmented cells and exceptionally large pigmented hair roots[1] has proved to be consistently dopa-negative in histological sections prepared according to several standard technical procedures[2,3]. A method utilizing photo-electric colorimetric evaluation of dopa-melanin has been evolved which permits comparison of this pigmented tissue with other tissues which can be shown by histological means to be dopa-positive.

Skin specimens were surgically removed, freed of hair and unpigmented skin, chilled to 4° C., minced in a few drops of phosphate buffer, and rapidly weighed on small squares of clean coverslip using a microtorsion balance. Increasing weights of minced tissues were placed in centrifuge tubes containing 2 ml. of freshly prepared 1:1000 1-dopa solution, buffered to pH 7.4, and thoroughly mixed. After four hours' incubation at 37° C. the tubes were centrifuged at 25,000 rpm. for 15 minutes. The supernatant fluid was decanted, diluted to a standard volume, and kept in chilled colorimeter tubes until read on a Klett-Summerson photo-electric colorimeter using a KS-42 filter. The auto-oxidative rate of dopa was determined for each experiment by means of a control tube containing only buffered dopa. The control tube was given a value of 100% and the values obtained for the different tissue samples were compared with it.

Using this technique, experiments were done on tissues from the Cloudman mouse melanoma and from human melanomas. These tumor tissues gave almost straight line curves with the activity (color formation) increasing directly with the weight of the tissue samples. The results agree favorably with those obtained from Warburg studies of the enzyme activity of such melanoma tissues[4].

A number of types of skin were studied by this method. These included skin from the neck, flank and pigment spot of the hamster, pigmented nipples from guinea pigs, neck and flank skin from mice, adult and infant human skin, including depigmented and hyper-pigmented negro skin. In contrast to the straight line activity curve of the tumor tissue, these skins showed a biphasic activity curve with the final inclination in the downward direction. This characteristic of pigmented skins has been interpreted to indicate the presence of inhibitory substances which interfere with the formation of dopa-melanin. This observation is in agreement with previous work showing such inhibitory substances to be present in the skins of guinea pigs[5], rabbits[6,7] and man[8,9].

When the conditions of the experiment were changed so that the weight of the tissue was held constant and the concentration, but not the volume, of the dopa solution was increased, a curve was obtained which is suggestive of the classic "substrate competition" curve. The addition of a sulfhydril substance, glutathione[10] increased the inhibition of color formation in dilute dopa solutions but did not significantly affect higher concentrations.

The effect of hormonal stimulation of pigmentation upon the ability of the stimulated skin to form dopa-melanin was investigated, using both the pigmented nipple of the guinea pig and the hamster pigment spot. In both instances, tissues which had been caused to hypertrophy and blacken under hormonal influence gave increased color formation with dopa as contrasted with untreated control tissues, but the essential biphasic shape of the curves was unchanged.

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Discussion by MORRIS FOSTER, Columbia University, New York.

Two important sources of error are inherent in the techniques used: (1) The colorimetric method, whereby pigment production rate is equated with oxidase activity. Such reasoning could be erroneous. For example, a reducing agent reacting with a substance produced after the oxidation of dopa could inhibit pigment production without affecting dopa oxidase activity; although, on the basis of colorimetric criteria, inhibition or lack of enzyme activity would be inferred. (2) The use of dopa as a substrate, since dopa can be oxidized via both the phenolase and cytochrome systems, whereas tyrosine is not affected by the latter. Thus enzymatic oxidation of dopa could be wholly or partly attributed to the cytochrome system rather than to the phenolase system. (See paper by Hesselback et al.)

A more direct measure of phenolase activity could be obtained by using tyrosine as the substrate and by measuring the rate of oxygen consumption in a Warburg respirometer.

In regard to the hamster pigment spot, an explanation of increasingly inhibited oxidative activity with increasing weight of pigment spot sample is difficult to make, since larger samples should also give larger amounts of enzyme, resulting in increased rather than decreased activity.

Reply by Shrader & Pfeiffer.

We would like to strongly emphazise that we do not know the enzyme system or systems involved in the production of dopa-melanin in these experiments. Our experiments were not designed primarily to elucidate the mechanisms of melanin formation but were intended to explain why the dopa reaction could not be obtained on sectioned hamster pigment spot. What we measured was the amount of dopa-melanin that was produced under experimental conditions as similar as possible to those of histological methods. The use of tyrosine as a substrate, or the Warburg apparatus as a method, would not have served to answer our original question. We do, however, feel that the results obtained justify the assumption that the colorimetric measurement of dopa-melanin is a valid criteria for an estimation of the pigment forming ability of the tissue.

In regard to the increase in inhibition which occurs as hamster pigment spot tissue weight is increased, we can only say that this is a characteristic of all skins tested and that we have postulated that it represents the results of some type of competitive action between the enzyme and inhibitor systems for utilization of the substrate. This suggested explanation gains further support from the fact that such inhibition is more marked when the quantity of available substrate is low and can be overcome by the addition of more substrate.

Fourth Session: Biochemical and Biophysical.

Introduction.

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Melanin chemistry may be roughly divided into two general approaches: (a) the character of the pigment noted in natural sources, i.e., the intracellular granules in skin, hair, plants, etc., and (b) the nature of the polymeric pigments obtained by the *in vitro* oxidation of such amino acids as tyrosine and dihydroxyphenylalanine, and the relation, if not the identity, of such pigments to those noted in natural sources. As yet, no clearcut